Doctoral (PhD) Dissertation

Theses

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Budapest

2025

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Multivariate analysis of factors influencing the development of plant pathogen infections

DOI: 10.54598/007180

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Introduction

Living organisms are not the simple receivers of environmental impacts, but they attempt to respond adaptively to the current environmental factors of their habitat. As a consequence of changes in climatic conditions, the geographical and temporal distributions of many species have been redrawn, shifting to new habitats that were previously unfavorable for them or generating new adaptive traits in crops or requiring novelties in production management. Vineyards are one of the most significant examples of these spreading crops, since most of the original wine-producing regions are located in the temperate climatic belt; however, in the last decades, vine grape has occupied new growing areas and has become nearly the largest volume produced crop in the tropics and subtropics area. Thus, the necessity for dynamic evolution is more evident in grape pathogens, as these fungi are influenced by environmental changes that accompany the changing production systems of grapevines.

At the microspatial scale (i.e., within a plant), plant pathogens are exposed to a wide range of organic materials, including airborne pollen grains and fungal spores, which can interact on the surface of grapes. The composition of the microbiome on the

plant surface is a dynamically changing system, characterized by spatial and temporal variability. The accumulated pollen could provide a species-specific nutrient supply for the initial fungal development on a plant surface.

At the large spatial scale (i.e., between vineyards) various environment-induced selection pressures could modify the occurrence of grape pathogen agents. There is strong evidence that vineyard features (e.g., row orientation), crop management (e.g., number of chemical treatments), soil properties (e.g., lime content), and grape variety features (e.g., ripening) influence the long-term prevalence of disease. Furthermore, local climatic conditions also act as a selection pressure on a large spatial scale. The local climatic conditions could generate shifts in grape phenology patterns, which could increase the risk of plant protection through exposure to phenology-dependent infection.

Therefore, to investigate the influencing factors of grape disease, it is necessary to amalgamate and integrate knowledge from the disciplines of plant production, plant biology, plant pathology, and environmental sciences. In this cross-disciplinary research series, we aimed to identify the key factors of a complex growing environment that define the long-term disease risk on both macro- and micro-spatial scales.

1. Pollen-spore interaction

Plant pollens and fungal spores are massively produced in natural habitats and large-scale agricultural areas, and the airborne transported biomass can be accumulated on relatively large crop surfaces. A pollen grain contains a specific chemical composition in quantity and quality, which is highly varied among plant species. So, the accumulated pollen could provide a species-specific nutrient supply for the initial fungal development in a pollen trap. Thus, this could increase the variation in pathogen infection risk in certain plants due to selection mechanisms such as host-pathogen coevolution, crop breeding, or pollination type. However, in pollen-spore interferences, the role of pollen-stimulated spore germination and species-specific differences is still less understood.

Therefore, we performed a multispecies experimental comparison (including 20 plant species) to test the role of taxon-specific attributes (i.e., host-pathogen compatibility, cultivation, pollination type, and pollen size) in the dose- and time-dependent responses of spore germination in *Botrytis cinerea*. We found a strong and general pollen-stimulating effect on spore germination; however, the triggering effect highly varied among plant species and differed between specific functional categories

across temporal and concentration gradients. Specifically, the *Botrytis cinerea* hosts, the non-cultivated plants and species with larger pollen sizes, increased the stimulation effects on spore germination, but not the pollination type. The systematic taxonomic and functional differences could be explained by the diversity of the pollens' chemical profile (i.e., composition matrix or specific trigger molecules), which different selection mechanisms might shape.

In crop-pathogen interactions, the pollen-stimulation effect could play a crucial role in early fungal development, increasing an additional risk to plant health in vineyards.

2. Effect of vineyard, management and variety characteristics on disease prevalence

Globalisation, climate change, and increasing consumer demands have forced the intensification of agricultural production. Thus, vineyards have expanded beyond the original production regions and into suboptimal areas, increasing the potential risk of disease outbreaks. Therefore, there is a rising need for a comprehensive and empirical revision of the interfering effects between grape infection and other external, large-scale factors such as environmental conditions and management practices. Although external abiotic and biotic

factors can determine the infection levels of grape disease in a complex way, existing studies focus on the short-term effects of only a single or a few potential factors.

In this large-scale study, we aimed to reveal the long-term impact of specific factors regarding vineyard characteristics, applied crop management and grape variety features, which could determine the infection severity of primary grape diseases, such as grey mould, downy mildew and powdery mildew, using a citizen science approach in Hungary, which is a traditional wine- and grape-producing country.

The present study has revealed that some vineyard properties (e.g., inclination, row orientation) and variety-specific features (e.g., bunch structure) were considered crucial from a plant protection aspect. At the same time, other factors were found to be less relevant in the present complex comparison, suggesting that the role of these factors might be overemphasised in the literature.

In conclusion, the susceptibility or tolerance of grapevines to pathogens appears to be an integrated effect of several factors and cannot be assigned to a single characteristic. The global changes urge the revision of conventional agricultural traditions

and deepen our knowledge about the infection process and pathogen-host-environment interactions.

3. Impact of vineyard properties on shifting grape phenology and its implications for plant diseases

Viticulture must adapt to global climate change, which may result in more frequent and extreme weather events. Additionally, innovative solutions and new management practices could also affect the microclimatic environment in a vineyard. The interfering environmental factors can be accelerated by the unfavourable weather events. Therefore, these effects could lead to shifts in grape phenological development, also known as a phenological shift. The less understood environment-driven grape phenological shift could make production even more challenging, as monitoring grapevine phenology is crucial for professional grape and wine producers due to the phenology-specific management decisions required. Hence, there was an increasing need for a thorough review of the phenological development based on existing Growing Degree Days (GDD) approaches, while also considering climatic factors and other environmental cues that affect vineyard management.

In this large-scale citizen science study, we aimed to identify the primary factors influencing the phenological shift of grape and explore the potential implications of this shift on the long-term severity of the globally important grape diseases, such as grey mould, downy mildew and powdery mildew.

We found that the single threshold GDD approach at an 8°C base temperature described grape development from fruit set to harvest most accurately. Among the vineyard properties, only the row orientation influenced grape phenology, resulting in a phenological shift. When studying the long-term occurrence of grape diseases, we found that various production-related factors influenced disease prevalence. However, climate and variety-driven grape development determined the prevalence of powdery mildew disease, but did not affect the prevalence of other pathogens.

This citizen science study provided insights into the role of the production environment in grapevine phenological development and into the function of the shifted phenology in the long-term disease occurrence. Changes in the global climate and the expansion of original production zones to suboptimal areas underscore the need to understand locally specific impacts in a

complex growing environment shaped by large-scale trends, which are crucial for climate-sensitive agricultural systems.

4. Long-term influences on disease occurrence: a meta-analytic comparison of vineyard, technology and soil features

Agricultural systems are constantly influenced by various environmental factors that create a complex production context, affecting plant conditions, crop yield, and quality. We can classify these environmental factors into three main groups, or "domains": Vineyard, Soil and Technology domains.

The Vineyard domain defines and incorporates variables related to vineyard characteristics, while the Soil domain encompasses variables regarding soil properties. The Technology domain refers to the specific agricultural technologies applied. These three domains cover the primary sets of factors that most significantly determine production outcomes in grape production.

Due to the simultaneous presence of these domains, the factors can reinforce or weaken each other. Therefore, our aims were to test 1) the overall effect on the relationships between long-term disease occurrence and relevant environmental variables 2) the

pathogen-specific roles in the overall effect and their heterogeneity, and 3) what type of environmental factors can affect disease occurrence in response to environmental factors in each grape pathogen?

We found that 1) the overall effect size of the complex growing context is low with a high heterogeneity level, 2) this heterogeneity showed a pathogen-specific patterns, 3) environmental variables have the most complex effect on BC, while PV and EN are only partially influenced by the growing environment, so their infection risk varies depending on a few specific similar features of the environmental factors (i.e., spatial and temporal scales). Specifically, among the pathogens, *Botrytis cinerea* is modified by the vineyard and soil domains, whereas we could not detect a significant impact of these conditions on *Plasmopara viticola* and *Erysiphe necator*. This suggests that the infections of these two pathogens are highly dependent on climatic variables.

These results highlighted how different results could be retrieved by examining variables within complex systems instead of testing them independently. Our study revealed that the long-term occurrence of grape primary pathogens originated from the cooperation of various interdisciplinary variable groups, known

as domains. We identified vineyard and soil domains as the main drivers of long-term disease occurrence. Using a comprehensive analytical approach, our study revealed that factors within the technology domain served a less relevant role compared to the other domains tested.

Summary

Agricultural systems are constantly under pressure from various environmental factors, creating a complex production environment. Crops and their pathogens are under similar adaptation processes, which are located within the same growing conditions over a long period, as in vineyards.

In our cross-disciplinary research series, we aimed to identify the most meaningful factors in complex growing environments which define the long-term disease prevalence of grape primary pathogens. To understand more about the complex effects of the growing environment, we have consistently used multivariate approaches and assessed their contributions at different spatial scales. This includes examining microscopic environments, with a focus on processes occurring in the phyllosphere, and also investigating the impact of large-scale global patterns on grape production at the country level.

This transdisciplinary research series provided comprehensive insights into the role of the production environment in plant protection strategy in grapevine production. We are committed to benefiting more from citizen science as a research and methodological approach, which enables us to combine the valuable experience of vine growers with the methodological knowledge of researchers, thereby increasing civil engagement and awareness of new scientific aspects. This approach offers an excellent opportunity to obtain large-scale data and provide comprehensive insights into current knowledge gaps in plant-pathogen-environment interactions.

New scientific results

8.1 Pollen-spore interaction

1. Using the Generalised Mixed Effect Model, I have proved that the aqueous extracts of pollen grains have a general stimulating effect on the germination of *Botrytis cinerea* conidia. The ratio of spore germination increases with time, along with the concentration gradient.
2. Species-specific functional attributes, such as host compatibility, cultivation and pollen size, highly affect the germination ratio of *Botrytis cinerea:* the efficiency of host pollen, non-crop plants and larger pollen grains is improved.

8.2 Vineyard, management and variety characteristics influence disease prevalence

3. Analysing the citizen science data using multivariate statistical models, I found that specific vineyard properties influence the long-term occurrence of *Botrytis cinerea* and *Plasmopara viticola*, but not *Erysiphe necator*. *Botrytis cinerea* infection is increased by the Northeast-Southwest row orientation, a higher proportion of adjacent plantations, a higher canopy wall, an increased number of chemical treatments and spatial variation. *Plasmopara viticola*

infection is more severe in areas with a higher inclination, row orientation, a higher proportion of adjacent plantations, and spatial variation.

4. Specific features of grape varieties influence the long-term occurrence of *Botrytis cinerea*: the occurrence of infection is increased by the delay in ripening periods, finer berry skin, and blanc berry colour.

8.3 Impact of vineyard properties on shifting grape phenology and its implications for plant diseases

5. Using a large-scale Citizen science survey, I revealed that the row orientation increased the grapevine phenological shifts along with the systematic deviation from the northern direction.
6. In *Erysiphe necator*, the infection occurrence is decreased with the increased phenological shift over the long-term.

8.4 Long-term influences on disease occurrence: a meta-analytic comparison of vineyard, technology and soil features.

7. Using a meta-analytical statistical approach on Citizen science data, I found that the complex growing context in viticulture, including environmental factors related to vineyard, soil, and technology domains, has a pathogen-

specific impact on overall effect sizes, which show consistently low overall effect sizes for each tested pathogen (*Botrytis cinerea*, *Plasmopara viticola*, *Erysiphe necator*) with low to high heterogeneity. These results suggest that the ongoing environmental factors' counterbalancing effects neutralise the opposing impacts, shaping the long-term presence of pathogens.

List of publications:

Journal articles with impact factor

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- Kocsis, Ivett; Petróczy, Marietta; Markó, Gábor (2023) Pollen-spóra interakció vizsgálata *Botrytis cinerea* kórokozón: Egy új megközelítés előzetes eredményei, GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 27: 1 (Supplement) pp. 188-194., 7 p.

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- Kocsis, Ivett; Petróczy, Marietta; Markó, Gábor (2020) Spóra-pollen interakció növénykórtani jelentőségének előzetes eredményei: A különböző növényi pollenek hatása a *Botrytis cinerea* konídiumainak csírázására, NÖVÉNYVÉDELEM 81. [N.S. 56]: 4 pp. 161-167., 7 p.

Further scientific articles

- Kocsis, Ivett; Ádám, János; Markó, Gábor (2019) A szürkepenész betegség növényvédelmi előrejelzése, AGROFÓRUM - A NÖVÉNYTERMESZTŐK ÉS NÖVÉNYVÉDŐK HAVILAPJA 30: 9 pp. 64-67., 4 p.

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- Kocsis, Ivett; Petróczy, Marietta; Markó, Gábor (2023) The role of vineyard characteristics, technology and cultivation in the grape diseases prevalence: first lectures of a large-scale citizen science In: ICPP 2023 Book of abstracts p. 1102
- Kocsis, Ivett; Petróczy, Marietta; Markó, Gábor (2020) A természetes pollencsapdák szerepe a szamóca és málna botrítiszes betegségének kialakulásában, In: Haltrich, Attila; Varga, Ákos (szerk.) 66. Növényvédelmi Tudományos Napok, Budapest, Magyarország: Magyar Növényvédelmi Társaság (2020) 103 p. p. 49, 1 p.
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